

# Laser-enhanced cavitation during high intensity focused ultrasound: An *in vivo* study

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Laser-enhanced cavitation during high intensity focused ultrasound (HIFU) was studied *in vivo* using a small animal model. Laser light was employed to illuminate the sample concurrently with HIFU radiation. The resulting cavitation was detected with a passive cavitation detector. The *in vivo* measurements were made under different combinations of HIFU treatment depths, laser wavelengths, and HIFU durations. The results demonstrated that concurrent light illumination during HIFU has the potential to enhance cavitation effect by reducing cavitation threshold *in vivo*. © 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4800780>]

High intensity focused ultrasound (HIFU) is a truly non-invasive thermal-ablation technique. HIFU works through rapidly depositing high intensity ultrasound energy into a small region to induce cell death primarily by hyperthermia after high intensity ultrasound is absorbed by soft tissue.<sup>1–5</sup> While the application of HIFU therapy is expanding, one concern related to HIFU treatment is the prolonged treatment time for large tumors because HIFU lesion is relatively small for each HIFU shot.

Cavitation has been shown to yield elevated heating rate above those produced by classical acoustic absorption in tissue and can provide an effective method to improve the efficiency of HIFU treatment.<sup>6–12</sup> However, pre-existing nucleation sites for cavitation are not omnipresent in most tissues *in vivo*. Many research efforts have been made to create nucleation sites for cavitation and reduce cavitation threshold. Both ultrasound contrast agents (UCAs)<sup>13–18</sup> and nanoparticles have been studied as methods to deliver cavitation nuclei into the targeted region.<sup>19,20</sup> The use of UCA or nanoparticles, however, requires the systematic injection of foreign particles into the blood stream, and would have major concerns regarding toxicity, efficiency, etc. Cavitation bubbles can also be induced in presonication areas by using low frequency, high intensity ultrasound prior to HIFU treatments.<sup>21,22</sup> This technique can enhance cavitation and create larger size lesions in deep tissue without the injection of any contrast agents. However, this technique requires very high acoustic pressure (generally more than 10 MPa) to be delivered into soft tissues in order to induce cavitation in the beginning. In addition, the inception of cavitation is erratic and difficult to predict when induced by ultrasound alone.

Laser light has been widely used as a reliable method to induce cavitation through optical breakdown. This procedure is generally performed with high intensity light, and mostly limited to clear media or sample surfaces.<sup>23,24</sup> Hence the application of this technique is limited in the

*in vivo* applications, where treatments in a certain depth in turbid media are often desired.

In a previous study,<sup>25</sup> we reported an enhanced heating effect during photoacoustic imaging-guided HIFU therapy. The results suggested that cavitation was enhanced when a diagnostic laser light beam illuminated the sample concurrently with HIFU radiation. Two features were highlighted in this previous study: (1) a diagnostic laser light beam was used, and the laser fluence was low and under the safety limit recommended by American National Standards Institute;<sup>26</sup> (2) cavitation was observed under the surface layer in a turbid medium. These results motivate us to further study the feasibility of laser-enhanced cavitation during HIFU, and test the limit of this technique.

In the current study, we further investigated laser-enhanced cavitation effect during HIFU in an *in vivo* experiment. Specifically, we investigated the effect of laser on cavitation threshold as a function of laser wavelength, HIFU duration, and treatment depth. The significance of this study lies in the fact that it may develop a technique to facilitate cavitation during HIFU with a diagnostic laser system; hence, HIFU heating can be enhanced without introducing foreign particles into the targeted tissue region.

A block diagram of experimental setup is shown in Fig. 1. A tunable optical parameter oscillator (OPO) laser (Surelite OPO PLUS, Continuum, Santa Clara, CA) pumped by a Q-switched, Nd:YAG laser with a pulse repetition rate of 10 Hz (5-ns pulse width) was employed as the irradiation source. The generated laser beam was formed into a ring-shaped illumination on a condenser lens, which was used to mount a 5-MHz HIFU transducer (SU-108-013, Sonic Concepts, Bothell, WA) (35 mm focal length and 33 mm aperture size) in the center hole. The condenser lens focused the laser beam underneath the HIFU transducer, and the optical focus overlapped with the ultrasound focus. A 10-MHz focused ultrasonic transducer (V315, Olympus-NDT, Waltham, MA) (37.5 mm focal length; 70%-6-dB fractional bandwidth), which was placed at a 90° with the HIFU transducer and acted as a passive cavitation detector (PCD), was aligned to be confocal with the HIFU transducer and the laser

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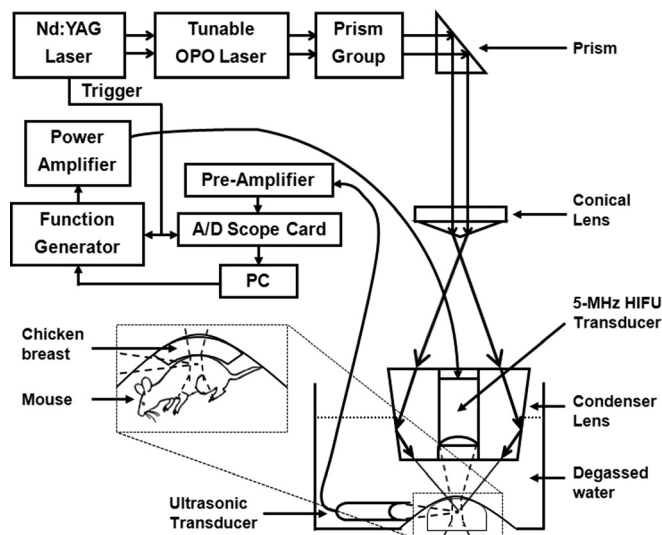


FIG. 1. System schematic.

beam prior to HIFU treatments. The signal detected by the PCD was directed to a pre-amplifier (5072PR; Olympus-NDT, Waltham, MA). Then, the resulting signals were captured by a data acquisition card (GageScope, CS21G8-256MSn Gage, Lockport, IL), and filtered by a 10-MHz high-pass filter to remove contributions from the HIFU fundamental and second harmonic frequencies in order to ensure that the detected signals were mainly received from broadband acoustic emissions of cavitation. Both the 10-MHz and 5-MHz transducers were immersed in a water tank that has a window on the bottom. The water tank was filled with degassed water, and the window at the bottom was sealed by a piece of polyethylene membrane.

During each experiment, the source signals were generated by a function generator (HP33250A, Agilent Technologies, Santa Clara, CA), amplified by a 50-dB radio frequency amplifier (350L, ENI Technology, Inc., Rochester, NY), and then sent to the HIFU transducer to generate HIFU waves with 95% duty cycle and 5 Hz repetition rate to ablate the tissue sample. In this study, we chose high duty cycle HIFU waves instead of continuous HIFU waves and implemented a 5% time window with HIFU off. The HIFU-off period can provide a time window for imaging in the future study. Additionally, HIFU signals was triggered by the laser system, which was running at a repetition rate of 10 Hz. Thus, in each HIFU burst cycle, only one laser pulse illuminated on the tissue sample surface when HIFU was on.

During the *in vivo* experiment, mice (BALB/c, 8-10 weeks old, female or male) were used. All animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Institutional Animal Care and Use Committee at the University of Kansas. Before each experiment, the animal was anesthetized with a mixture of ketamine (87 mg/kg body weight) and xylazine (13 mg/kg body weight) and shaved in the leg region. The shaving procedure included the use of standard surgical hair removal lotion because hairs interfere with ultrasound propagation. After shaving, the animal was maintained under anesthesia with an isoflurane gas

anesthesia machine (1%–2% isoflurane in pure oxygen) for at least 1 h to allow the animal to reach equilibrium body condition. Surgical tapes were used to gently secure the animal on a warm pad. A custom-designed animal holder was also used to fix the animal with ear-pins and a tooth-pin. The animal was then secured underneath the membrane at the bottom of the water tank for subsequent experiment. Ultrasound coupling gel was applied to the top surface of the sample to provide coupling between the membrane and the sample surface. Heart-rate and blood oxygenation were monitored with a pulse-oximeter during the experiment, and breathing was visually monitored.

During the experiment, different combinations of laser intensities and ultrasound pressures were applied to the leg muscle of the animal, and the generated cavitation signals were detected by the PCD to determine the pressure thresholds<sup>8</sup> for cavitation. The cavitation threshold measurements were repeated for 5 times at different locations on the animal leg area for the standard deviation calculation. Laser wavelengths in near-infrared (NIR) region such as 760 and 960 nm were used in order to achieve deep penetration depth.

The corresponding HIFU focal pressure in the tissue was obtained from a finite difference time domain (FDTD)<sup>27</sup> algorithm using acoustical properties of the tissue sample (1540 m/s and 0.3 Np/cm at 5 MHz). The laser fluence was first measured at the surface of the tissue sample, and the mean of the Monte Carlo (MC) method was used to estimate the laser fluence inside the tissue.<sup>28</sup>

In order to test the capability of generating cavitation at different depths, we used *ex vivo* chicken breast tissues with different thicknesses to cover the region of interest on the small animal (Fig. 1 dotted line circled area). In order to avoid cavitation on the interface, ultrasound coupling gel was not used since the air in the gel would promote the occurrence of cavitation. Alternatively, degassed water was used as the coupling medium, which is much better than using ultrasound gel because no strong cavitation signals are detected from the interface. Also, during the experiment, the confocal point of the transducers and the laser was carefully aligned into a leg of the small animal, which was  $\sim 1$  mm under the skin. The total treatment depths were 5 mm and 10 mm from the top surface of the sample with the thickness of chicken breast tissue of 4 mm and 9 mm, respectively.

Fig. 2(a) shows the measured cavitation threshold at 5-mm depth when the laser wavelength was 760 nm with a 2-s HIFU sonication. The result shows that the cavitation threshold decreased as the laser fluence increased. When no laser was applied, the detected cavitation threshold was 9.80 MPa. When the surface laser fluence was increased to 50 mJ/cm<sup>2</sup>, which corresponded to a fluence of 13.6 mJ/cm<sup>2</sup> in the HIFU focal region by Monte Carlo simulation, the measured cavitation threshold was reduced to 7.89 MPa. An example of the cavitation signals received by the PCD is shown in Fig. 3. We observed that very weak cavitation signals were detected by the PCD when there was no laser. However, cavitation acoustic emissions were clearly detected while combining the HIFU treatment with the laser irradiation on the tissue sample. Fig. 2(b) shows the results with the similar parameter settings but the HIFU treatment depth was 10 mm. At this

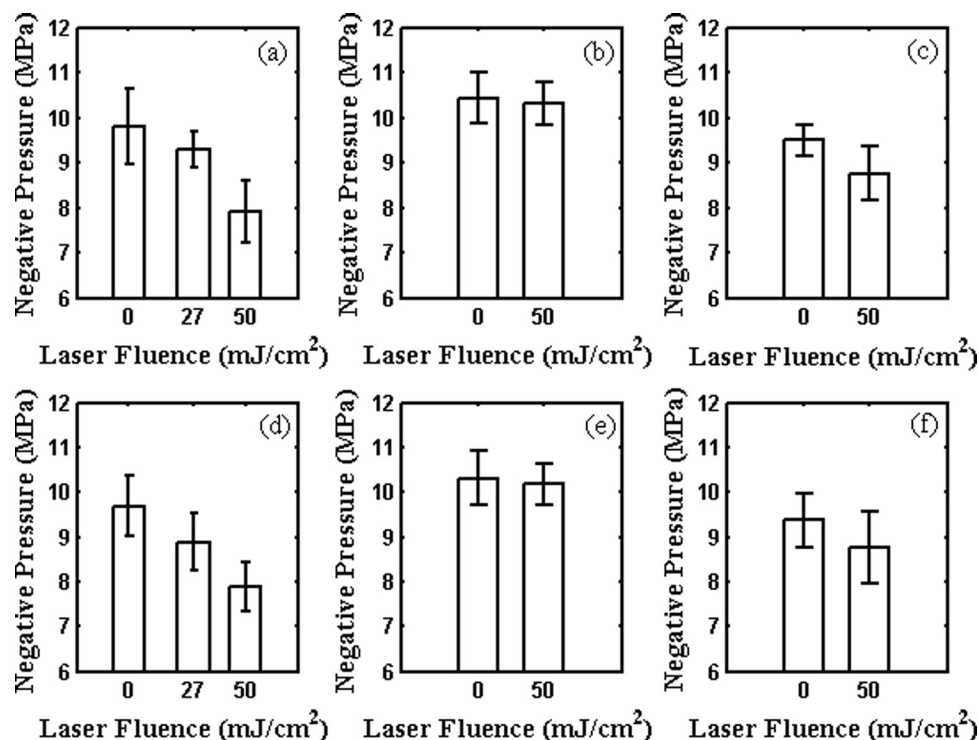


FIG. 2. *In vivo* measurements of cavitation pressure threshold. The mean acoustic cavitation thresholds from five measurements were plotted as a function of laser fluence at the sample surface. Error bars are the standard deviations of five measurements. (a) 760-nm laser wavelength, 5-mm treatment depth, and 2-s HIFU duration time. (b) 760-nm laser wavelength, 10-mm treatment depth, and 2-s HIFU duration time. (c) 760-nm laser wavelength, 10-mm treatment depth, and 4-s HIFU duration time. (d) 960-nm laser wavelength, 5-mm treatment depth, and 2-s HIFU duration time. (e) 960-nm laser wavelength, 10-mm treatment depth, and 2-s HIFU duration time. (f) 960-nm laser wavelength, 10-mm treatment depth, and 4-s HIFU duration time.

depth, the measured cavitation threshold was similar between with and without laser (10.43 MPa versus 10.31 MPa). We estimated that the laser fluence at the HIFU focal spot was only  $3.6 \text{ mJ/cm}^2$ , which was very low and might explain the reason why laser had little effects on enhancing cavitation. Fig. 2(c) shows the measured cavitation thresholds at 10-mm depth with 760-nm laser wavelength and a 4-s HIFU sonication. When laser light was applied, the cavitation threshold was reduced from 9.50 MPa to 8.76 MPa. As compared with Fig. 2(b), whereas the only difference was the duration of HIFU sonication, Fig. 2(c) shows that laser-enhanced cavitation could be facilitated by longer HIFU sonication durations.

The above cavitation threshold measurements were repeated at 960-nm laser wavelength with the same HIFU

and laser parameters. At this laser wavelength, the treatment depth and HIFU duration had the similar influence on the cavitation threshold as that at 760-nm laser wavelength. However, as compared with Figs. 2(a)–2(c), Figs. 2(d)–2(f) show that using laser wavelength 960-nm further reduced the cavitation threshold, although the differences were in the range of error. The major difficulty at this depth was that the laser fluence dropped to a very low level, and therefore, the enhancement effect on cavitation threshold became low. However, longer wavelength lights should have advantages to enhance cavitation in the deep region because that as the laser wavelength increases in NIR region, light can penetrate deeper, and therefore retain more energy in a certain treatment depth.<sup>29–31</sup>

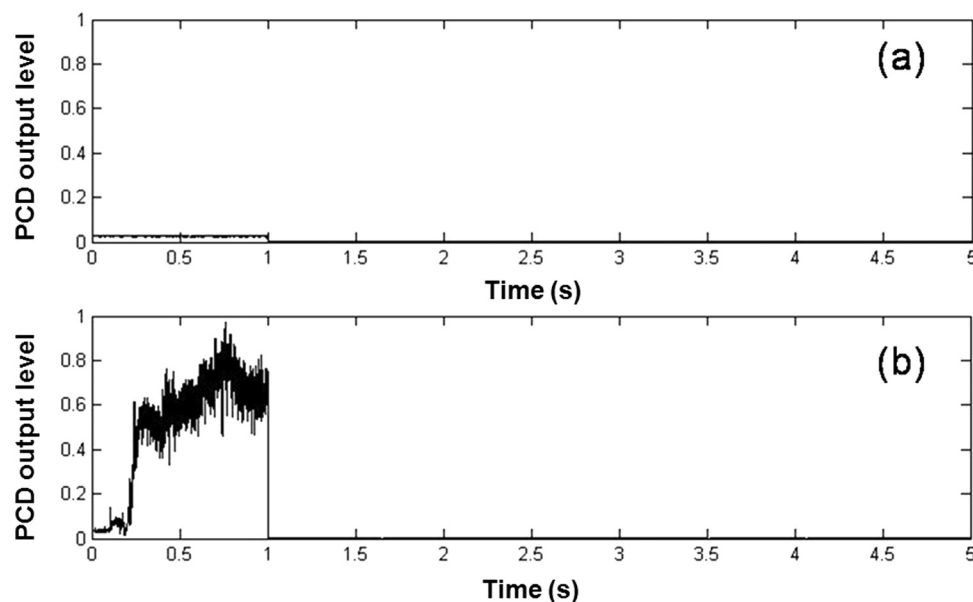


FIG. 3. Cavitation signals detected by PCD as a function of time without (a) and with (b) laser. Laser wavelength and laser fluence on the sample surface was 760 nm and  $27 \text{ mJ/cm}^2$ , respectively. HIFU treatment depth was 5 mm. HIFU pressure was 10.16 MPa.

In this study, we showed *in vivo* results for laser-enhanced cavitation effect during HIFU. The results suggest that cavitation effect can be enhanced when laser light is applied to the sample during HIFU sonications. The magnitude of the enhancement, however, seems related to the applied laser fluence. The enhancement will be greater if the applied laser fluence is higher. Both laser wavelength and HIFU duration can also affect the detected cavitation threshold. The major limitation of this technique will be the depth because light is strongly scattered in soft tissue.

Laser light is well-known for its ability to nucleate cavitation through vaporization.<sup>24,32</sup> To vaporize the surrounding fluid through either optical absorption or optical breakdown, high optical intensity is needed. In our results, however, the laser fluence was under the safety limit recommended by the American National Standards Institute,<sup>26</sup> and suggested that significant heating or chemical breakdown by laser alone is unlikely. Therefore, the combination of ultrasound and laser is the key for cavitation. A possible mechanism is that there is an instantaneous heating in the nano-second scale during laser illumination. When this instantaneous heating is coupled with negative ultrasound pressure, cavitation will be induced.

In summary, concurrent light illumination during HIFU has the potential to enhance cavitation by reducing cavitation threshold. In comparison with the other methods to enhance HIFU, this technique does not involve the use of any nanoparticles or ultrasound contrast agents.

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